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BET1L and *TNRC6B* associate with uterine fibroid risk among European Americans

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Abstract

Uterine fibroid (UFs) affect 77% of women by menopause and account for $9.4 billion in healthcare costs each year. Although UFs are heritable, genetic risk is poorly understood. The first genome-wide association study (GWAS) of UFs was recently performed in a Japanese population, with reported genome-wide significance for single nucleotide polymorphisms (SNPs) across three chromosomal regions. We tested these SNPs for association with UFs in U.S. cohorts. Women were enrolled in the *Right from the Start* (RFTS) cohort and the BioVU DNA repository. UF status in both cohorts was determined by pelvic imaging. We tested 65 candidate and haplotype-tagging SNPs for association with UFs presence using logistic regression in RFTS and the top three GWAS associated SNPs in BioVU. We also combined association results from both cohorts using meta-analysis. 1,086 European American (EA) cases and 1,549 controls were examined. Two SNP associations replicated (blocked early in transport 1 homolog[*BET1L*] rs2280543, RFTS-BioVU meta-odds ratio[OR]=0.67 95% confidence interval[CI] 0.38 to 0.96, Q=0.70, I=0, $p=6.9\times10^{-3}$; trinucleotide repeat containing 6B[*TNRC6B*] rs12484776, RFTS-BioVU meta-OR=1.21, 95% CI 1.07 to 1.35, Q=0.24, I=28.37, $p=8.7\times10^{-3}$). Meta-analyses combining evidence from RFTS, BioVU, and prior GWAS showed little heterogeneity in effect sizes across studies, with meta-p-values between 7.45×10^{-8} to 3.89×10^{-9}, which were stronger than prior GWAS and supported associations observed for all previously identified loci. These data suggest common variants increase risk for UF in both EA and Japanese populations. However, further research is needed to assess the role of these genes across other racial groups.

Keywords

Uterine leiomyoma; fibroids; genetic epidemiology; polymorphism; women's health

INTRODUCTION

Uterine leiomyomata, or fibroids (UFs), are the most common female pelvic tumor. Prevalence estimates range from 20% to 77%, increasing with age up to menopause.(Cramer

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and Patel, 1990;Marshall et al., 1997;Vollenhoven, 1998) Known risk factors for UFs include African American (AA) race,(Baird et al., 2003;Cramer and Patel, 1990;Faerstein et al., 2001;Marshall et al., 1997;Ojeda, 1979) early age-at-menarche, (Dragomir et al., 2010;Faerstein et al., 2001;Lumbiganon et al., 1996;Marshall et al., 1998;Samadi et al., 1996;Wise et al., 2004) high body mass index (BMI),(Moore et al., 2008;Takeda et al., 2008) and increased age.(Baird et al., 2003) In addition, a protective effect for UFs has also been observed with higher parity, likely due to pregnancy-related hormonal and physical changes including postpartum uterine involution.(Baird and Dunson, 2003;Laughlin et al., 2010a;Laughlin et al., 2011)

Multiple lines of evidence have shown that UFs are influenced by genetic risk factors. First, UFs are highly heritable with evidence from twin-pair and familial aggregation studies. (Luoto et al., 2000;Treloar et al., 1992) Heritability studies of UFs in several European populations have observed that between 26 and 69% of UF risk is due to genetic factors. (Kurbanova et al., 1989;Luoto et al., 2000;Snieder et al., 1998) Further supporting a genetic contribution to risk are the observed racial disparities in UF age of onset, number, size, and lifetime incidence by menopause.(Baird et al., 2003) Genetic epidemiology studies to date have been largely limited to small-scale or single marker studies of steroid hormones, particularly estrogen, as it is potentially the most critical regulator of fibroid growth.(Flake et al., 2003) Also other growth factors,(Sozen and Arici, 2002) reproductive factors, (Parazzini et al., 1996) dysregulation of microRNAs,(Marsh et al., 2008) shortening of telomeres,(Bonatz et al., 1998) excessive production of disorganized extracellular matrix, (Malik et al., 2010;Sozen and Arici, 2002) and acquired chromosomal aberrations have been noted in UF studies.(El-Gharib and Elsobky, 2010)

Recently a genome-wide association study (GWAS) by Cha and colleagues appeared in *Nature Genetics* that examined risk for UFs among a population of Japanese women.(Cha et al., 2011) Eleven single nucleotide polymorphisms (SNPs) in three chromosomal regions (10q24.33, 11p15.5, and 22q13.1) associated with increased risk of UFs. The SNPs identified in the GWAS mapped to or nearby the genes STE20-like kinase (*SLK*), oligonucleotide/oligosaccharide-binding fold containing 1 (*OBFC1*), trinucleotide repeat containing 6B (*TNRC6B*), outer dense fiber of sperm tails 3 (*ODF3*), blocked early in transport 1 homolog (*BET1L*), resistance to inhibitors of cholinesterase 8 homolog A (*RIC8A*), and sirtuin 3 (*SIRT3*). The degree to which these findings generalize across racial groups is unknown, as they have not been replicated in non-Asian populations. Based on findings from other complex diseases, the same genetic factors may not explain UF risk across racial groups. Furthermore, since this is a clinical cohort is may be that these SNPs are more associated with severe and/or symptomatic fibroids. To date there are no published GWAS of UFs in U.S. populations.

In efforts to examine the three chromosome regions more comprehensively for an association with UF risk, we examined haplotype-tagging and index SNPs in a European American (EA) U.S. population rather than limiting our selection to only the previously studied variants. To conduct this analysis we used two cohorts of women, all of whom had pelvic imaging performed to detect the presence of UFs. Imaging is critical, because many women with UFs are asymptomatic and without imaging, studies may misclassify as many as 51% of women.(Baird et al., 2003;Myers et al., 2012) The primary goal of this study was to determine if gene variants within the previously associated gene regions associate with UF risk in an independent U.S. population.

MATERIALS AND METHODS

Study Populations

***Right from the Start* (RFTS)—**RFTS is a community-based pregnancy cohort that enrolled study participants between 2001 and 2012. RFTS enrolled participants from Galveston, Texas; Memphis, Nashville, Knoxville, and Chattanooga, Tennessee; and the Research Triangle region (Raleigh, Durham, and Chapel Hill) in North Carolina. These analyses included RFTS participants who were 18 years or older and non-Hispanic EAs. As a part of participation, consent was obtained to review study participant medical records. Direct marketing and recruitment strategies have been previously described.(Promislow et al., 2004) The institutional review board (IRB) of Vanderbilt University, Nashville, Tennessee approved this study.

At enrollment, a research transvaginal ultrasound was conducted to assess embryonic development for the study pregnancy and to systematically examine the uterus for presence of UFs. The fibroid measurement protocol required three separate sets of measurements for each UF, with assessment of three perpendicular diameters: length, width, and depth. RFTS includes fibroids as small as 0.5 centimeters (cm) in maximum diameter.(Laughlin et al., 2009) Multiple still images of each UF with caliper markings of each diameter were recorded and a UF map was completed indicating the location and type of all UF(s).

Participants completed an intake interview at enrollment and a computer assisted telephone interview at the end of the first trimester. The intake and first trimester interviews provided information on reproductive history and candidate confounders. DNA samples were obtained either in person or by mail during follow-up using Oragene saliva DNA kits (DNA Genotek Inc., Ontario, Canada).

The BioVU DNA Repository—The BioVU Repository (2007 – present) is located at Vanderbilt University, Nashville, TN and was designed to link clinical data available from de-identified electronic medical records to DNA specimens.(Pulley et al., 2010) The BioVU Repository consists of de-identified blood samples obtained from patients at Vanderbilt University Medical Center Hospital, including all clinics that are part of the hospital system. De-identified data from multiple sources are available within BioVU, including diagnostic and procedure codes, basic demographics, discharge summaries, nursing notes, progress notes, health history, multi-disciplinary assessments, laboratory values, echocardiogram diagnoses, imaging reports, electronically derived data, and inpatient medication orders. All subjects (both UF cases and controls) selected from BioVU had diagnostic imaging with ultrasound, magnetic resonance imaging (MRI), or computed tomography (CT). Included as UF cases were women who had diagnostic imaging and either a diagnosis of a UF, as indicated by physician diagnosis of UFs or a surgical procedure for UF removal. For controls, two or more instances of pelvic imaging on separate dates were required. Initial chart review of a small subset of controls suggests that a large proportion of imagining information comes from prior pregnancy ultrasounds. Women with hysterectomy, myomectomy, or other procedures for UFs were excluded as controls. Controls were density matched to UF cases based on date of diagnostic imaging, where controls second imaging date had to be within a three to five year window of those cases. Both cases and controls were 18 to 65 years of age. We did not limit controls for age, but did perform secondary analyses limiting controls to those greater than 50 years of age to reduce the possibility that some women might develop a UF after imaging was performed. Our sampling algorithm to define UF cases and controls is informed by a published UF algorithm by Hartmann and colleagues using electronic medical records.(Hartmann et al., 2006) The IRB of Vanderbilt University, Nashville, TN approved this study.

SNP Selection

SNPs were selected based on either previously being associated with UFs in the GWAS by Cha and colleagues or being a haplotype-tagging SNP.(Cha et al., 2011) The SNPs with the strongest associations by Cha and colleagues were rs2280543 (chromosome 11, in the *BET1L* gene), rs12484776 (chromosome 22, in the *TNRC6B* gene), and rs7913069 (chromosome 10, not located within a gene, but referred to as "nearby SLK"). The remaining SNPs selected were haplotype-tagging SNPs near these loci. Haplotype-tagging SNPs were identified using the HapMap phase III samples (Release 28, http://www.hapmap.org): African American (from the ASW USA), Yoruban from Ibadan, Nigeria (YRI), and Northern and Western European (Centre d'Etude du Polymorphisme Humain (CEPH) family samples from Utah, USA), with the Tagster htSNP linkage disequilibrium (LD) selection tool available from the SNPinfo Web Server (National Institute for Environmental Health Sciences; http://snpinfo.niehs.nih.gov/). Within each reference population selected SNPs had a minor allele frequency (MAF) of 0.10 or greater, were in bins of highly correlated SNPs (r^2 greater than or equal to 0.80), and were located within five kilobases from the boundaries of candidate genes and/or SNPs if the index SNP was not located within a gene. The GWAS index SNPs were forced into the Tagster htSNP selection algorithm and through an iterative approach, a minimum set of htSNPs for study subjects with admixed ancestry were identified.(Thorisson et al., 2005;Xu et al., 2007) A total of 72 SNPs met the above criteria. A summary of the SNPs used in our final analyses is provided in Table 1 and gene schematics are provided on Supplemental Figures 1 through 3. Information regarding SNP location within a gene, its type, and any corresponding amino acid changes (none were found) were sought from the HapMap and the SNPper program (http://snpper.chip.org/).

DNA Extraction and Genotyping

Genotyping BioVU—BioVU DNA samples were isolated from whole blood using the Autopure LS system (QIAGEN Inc., Valencia, CA). In BioVU we only genotyped the top three associated SNPs from the previously published GWAS (rs7913069, rs2280543, and rs12484776) and they were all genotyped using a TaqMan allelic discrimination assay.

RFTS Genotyping—DNA for RFTS saliva samples was extracted using Oragene DNA (Genotek Inc., Ontario, Canada) manufacturer recommended DNA extraction procedures. In the RFTS population, one tag SNP (rs6519215) was genotyped using a TaqMan allelic discrimination assay purchased from the ABI Assay on Demand or Assays by Design services (Life Technologies, Grand Island, NY) . The remaining 71 SNPs were genotyped using the Sequenom MassARRAY genotyping platform (Sequenom Inc., San Diego, CA). One SNP (rs5757906) assay failed. The final analytic dataset for RFTS contained 65 SNPs. All SNPs in BioVU and RFTS had genotyping call rates of 95% or better (mean call rates of 98%) and QC sample match rates of 100%. Six SNPs were dropped because of low MAF (< 0.01) in the genotyped dataset.

Statistical Analysis

Tests for deviations from Hardy Weinberg Equilibrium (HWE) were performed using PLINK statistical software.(Purcell et al., 2007) Statistical significance for these analyses was determined using p values from Fisher's exact tests. Pairwise LD was characterized using the standard summary statistic r^2 from HaploView(Barrett et al., 2005) statistical software, where r^2 is the correlation of SNPs in a population that takes into account differences in allele frequencies and is less sensitive to inflation due to small sample size. Haplotype blocks were assigned, using the D' confidence interval algorithm created by Gabriel et al.(Gabriel et al., 2002) Descriptive statistics of demographic data were expressed

as frequencies and proportions and compared between women with and without UFs (reference) using unadjusted logistic regression using STATA 11.0 statistical software (College Station, TX).

Single locus tests of association with UF risk were performed using logistic regression assuming an additive genotypic model (0 (homozygous major allele) versus 1 (heterozygous) versus 2 (homozygous minor allele)). Odds ratios (ORs) and confidence intervals (CI) were reported for SNPs from all statistical models. We reported results from both regression models unadjusted and adjusted for potential confounders: age (categorical) and BMI (categorical). Unadjusted models are presented in the manuscript for comparison with the previous results from Cha and colleagues; adjusted models can be found in Supplemental Table 1. PLINK statistical software was used to perform single locus tests of association.(Purcell et al., 2007)

Single locus association analyses in RFTS and BioVU were further analyzed together with fixed-effects meta-analyses using PLINK as well as METAL.(Purcell et al., 2007;Willer et al., 2010) We only considered the fixed effects results among EAs from RFTS and BioVU. Thereby, we sought out only those loci with consistent evidence between the two populations using this approach.

RESULTS

Right from the Start (RFTS)

Fourteen percent of women from RFTS had UFs (n=89). Age greater than or equal to 30 years was associated with increased risk for UFs (Table 2A). None of the SNPs 65 haplotype-tagging SNPs examined significantly deviated from HWE. In unadjusted analyses, five SNPs associated ($p \leq 0.05$) with increased risk of UFs among EAs (Table 3). Among these associated SNPs, one was in the 10q24.33 chromosomal region (rs11191875, OR = 2.78, 95% CI 1.24 to 6.26, p = 0.014), one was in *BET1L* (rs939917, OR = 1.86, 95% CI 1.12 to 3.07, p = 0.016;) and three were in *TNRC6B* (rs11089974, OR = 1.46, 95% CI 1.01 to 2.10, p = 0.046; rs12484776, OR = 1.48, 95% CI 1.03 to 2.13, p = 0.035; rs4821942, OR = 1.52, 95% CI 1.06 to 2.18, p = 0.024). The set of SNPs that were associated with UFs also showed evidence for association after adjustment for age and BMI (Supplemental Table 1).

Further examination of the LD structure among the SNPs in *TNRC6B* that associated with UF risk showed evidence for high LD between rs11089974, rs12484776, and rs4821942—with r^2 values between these SNPs ranging from 0.88 to 0.97 in cases and 0.87 to 0.93 in controls (Supplemental Figure 4). Based on the strong LD observed between these *TNRC6B* SNPs, we performed single SNP association analyses conditioning on rs12484776 (GWAS index SNP).These analyses showed that none of the SNPs in *TNRC6B* were statistically significant after adjusting for rs12484776 in regression models (results not shown). This would suggest that associations at other SNPs in *TNRC6B* were due to being in LD with rs12484776.

BioVU

BioVU participants were on average older than RFTS study participants (Table 2). BioVU genotyping data were only for the top three previously associated GWAS SNPs. Fifty percent of women included in these analyses from BioVU had UFs. Similar to women from RFTS, older age was associated with increased risk for UFs (Table 2B). Greater proportions of women from BioVU had higher BMIs or were older than women in RFTS; this reflects RFTS samples coming from a younger cohort while BioVU represents a clinical population.

None of the SNPs examined significantly deviated from HWE. Among the three index SNPs examined for association with UF risk, two showed evidence for association in unadjusted analyses (*BET1L* rs2280543 OR = 0.68, 95% CI 0.51 to 0.92, p = 0.013; *TNRC6B* rs12484776 OR = 1.17, 95% CI 1.00 to 1.36, p = 0.050) (Table 3). This evidence for association with UFs remained after adjusting for age and BMI (Supplemental Table 1).

We were not able to determine the age at which a study participant developed UFs or if they develop a UF after being screened. To address this in BioVU we performed secondary analyses limiting BioVU controls to women over 50 (data not shown). Risk estimates for UF were larger when limiting BioVU controls to women over 50 (*BET1L* rs2280543 OR = 0.71, 95% CI 0.51 to 0.99, p = 0.042; *TNRC6B* rs12484776 OR = 1.26, 95% CI 1.05 to 1.51, p = 0.011) suggesting that despite observing consistent associations at index SNPs, the younger subset of controls may have been contributing to phenotypic heterogeneity.

RFTS BioVU meta-analyses

Meta-analyses across RFTS and BioVU samples showed strong evidence of association at *BET1L* rs2280543 (meta OR = 0.67, SE = 0.15, Q = 0.70, I = 0, $p = 6.90\times10^{-3}$) and *TNRC6B* rs12484776 (meta OR = 1.21, SE = 0.07, Q = 0.24, I = 28.37, $p = 8.70\times10^{-3}$) (Table 4). Finally, in order to assess the consistency of effect sizes and association results with the prior GWAS of a Japanese population, we did a meta-analysis including all RFTS participants, BioVU participants, and the prior Japanese GWAS (Table 4). Statistical significance was stronger for all three SNPs compared to the level of significance in the prior GWAS. Little evidence of heterogeneity across the study populations was indicated for these SNPs, with Q's ranging from 0.21 to 0.92 and I = 0 to 36.01. The SNP with the strongest meta-association p value across all populations in RFTS, BioVU, and the prior GWAS of Japanese subjects was *BET1L* rs2280543 (OR = 0.66, SE = 0.07, Q = 0.92, I = 0, $p = 3.89\times10^{-9}$), which associated with $p = 7.16\times10^{-7}$ in the paper by Cha and colleagues. (Cha et al., 2011) This level of statistical significance exceeds the canonical genome-wide threshold for multiple testing, using a Bonferroni correction for multiple testing. It is of note, however, that in the prior GWAS they used the major allele as the risk allele for rs2280543. We used the minor allele as the risk allele in our analyses and our results are consistent for rs2280543 when modeled with the same risk allele.

DISCUSSION

This study is the first replication of the associations previously observed in *BET1L* and *TNRC6B* in two EA U.S. cohorts and is enhanced by pelvic imaging for cases and controls. We observed strong evidence of association across several markers in *BET1L* and *TNRC6B* including two of the previously associated GWAS index SNPs. The strongest evidence for association came from our EA subset; however, we were underpowered to detect associations across the other racial groups. The direction of the effect sizes across SNPs in the prior Japanese GWAS and our study were consistent with little evidence of heterogeneity in effect sizes across studies. The very low heterogeneity of effects at these loci between European and Asian populations further support a consistent effect on risk and suggest that this locus may be functional or in tight LD with the functional SNP. We did not replicate the association previously observed at rs7913069 within RFTS or BioVU; however, the SNP was significant when we meta-analyzed including the prior GWAS with a higher level of statistical significance than was previously reported (GWAS $p = 7.9\times10^{-8}$). We note, however, we were less powered to detect an association among EAs at this SNP because the MAF was 0.01 while among the Japanese population the MAF was between 0.07 and 0.11.(Cha et al., 2011)

We note that the associations at the SNPs identified by Cha and colleagues did not previously replicate in a cohort of African American women from the *Black Women's Health Study* (BWHS) and were not among the top associations reported in a recent association study using women of European ancestry (U.S. European Americans and Australians).(Eggert et al., 2012;Wise et al., 2012) Inconsistencies in association results between our study and these previously published studies may be due to the genetic ancestry of the study participants, as all of our participants were EAs from the U.S. while the prior two studies consisted of African Americans and combined subjects of European ancestry from the U.S. and Australia. It may be that these SNPs associate among EAs from the U.S. and Japanese populations but not other racial or geographic groups. Furthermore, the phenotype definition used to define cases and controls by these prior studies was based on self-report, while our studies required imagining confirmation of fibroid status.

The strongest evidence for association with UF came from *BET1L* and *TNRC6B*. Neither *BET1L* nor *TNRC6B* were previously associated with UF risk, except for the GWAS by Cha and colleagues. According to the NHGRI Catalog of Published GWAS (http://www.genome.gov/gwastudies/), the *BET1L* SNP rs2280543 has also been associated with intracranial aneurysm in another GWAS in a Japanese population.(Low et al., 2012) Although rs12484776 has not been identified by other GWAS, other SNPs within the *TNRC6B* have been associated with both prostate cancer risk among EA and height.(Estrada et al., 2009;Liu et al., 2011;Sun et al., 2009;Tao et al., 2012) *TNRC6B* has been shown to interact with insulin-like growth factors 2 (*IGF-2*) *to* increase risk for prostate cancer.(Tao et al., 2012) Furthermore, quantitative trait loci within the region of *TNRC6B* have been shown to be associated with age-at-menarche and early age-at-menarche is an established risk factor for UF.(Dragomir et al., 2010;Faerstein et al., 2001;Guo et al., 2006;Lumbiganon et al., 1996;Marshall et al., 1998;Samadi et al., 1996;Wise et al., 2004) *BET1L* is involved in endoplasmic reticulum to golgi transport while *TNRC6B* is involved in RNA interference machinery and is important for miRNA RNA-dependent translational regression or degradation of target RNAs. *TNRC6B* is a potential biological target as miRNAs have previously implicated in leiomyoma pathogenesis.(Luo and Chegini, 2008;Meister et al., 2005)

Further examination of the genes near the GWAS index SNPs show strong evidence of those genes being involved in cardiovascular-related health conditions. *OBFC1* has been associated with cardiovascular disease and *SIRT3* with metabolic syndrome, mitochondrial function, obesity, and exercise response in prior studies.(Borengasser et al., 2011;Burnett-Hartman et al., 2012;Capel et al., 2008;Choudhury et al., 2011;Giralt and Villarroya, 2012;Green and Hirschey, 2012;Guarente, 2011;Mestre-Alfaro et al., 2012;Valdecantos et al., 2012;Vasan et al., 2007) Insertion/deletions within the *BET1L* chromosomal region have also been implicated in glucose regulation and type II diabetes.(Owerbach et al., 1982;Rotwein et al., 1981) These data suggest that genes associated with metabolic complications and cancer may also be involved with UF pathogenesis, which is interesting as being overweight is a risk factor for UFs.(Baird et al., 2007;Takeda et al., 2008;Terry et al., 2007;Wise et al., 2005) Further research is necessary to assess the possible role of genetic interactions with cardiovascular outcomes in UF risk.

There are no other established genetic risk factors for UFs. In addition to the recently published GWAS by Cha and colleagues(Cha et al., 2011) there have been three other prominently published large-scale genetic association studies.(Eggert et al., 2012;Makinen et al., 2011;Wise et al., 2012) These include a tumor sequencing study published by Mäkinen and colleagues published in the journal *Science*,(Makinen et al., 2011) a GWAS of UF using a EA family and population-based sample,(Eggert et al., 2012) and an admixture mapping analysis using a African American populations.(Wise et al., 2012) Among these

only the study by Mäkinen and colleagues has been validated in multiple independent studies. The Eggert study observed one locus at genome-wide significance, but without an independent replication. Mäkinen and colleagues examined somatic mutations in tumor tissue and found most UFs had mutations at the gene mediator complex subunit 12 (*MED12*), a result that has replicated across independent multi-ethnic populations.(Je et al., 2012;Makinen et al., 2011) *MED12* is a 26-subunit transcriptional regulator that bridges DNA regulatory sequences to the RNA polymerase II initiation complex. All associated mutations resided in exon 2, suggesting that aberrant function of this region of *MED12* contributes to tumorigenesis. Although some recent research suggests that mutations in *MED12* are specific to UF tissue,(Je et al., 2012) other studies suggest that *MED12* may be involved in multiple pathways that contribute to tumor growth in other tissues.(Markowski et al., 2012) Further supporting the later hypothesis is a recent study published in *Nature Genetics* showing that *MED12* mutations are also present in prostate cancer tumor tissue. (Barbieri et al., 2012) Further research can elucidate any relationship *MED12* may have with the genes identified by Cha and colleagues.(30)

A significant strength of our study is that all women were systematically screened for UFs using a standardized protocol and endovaginal ultrasounds for RFTS and various forms of pelvic imaging for BioVU. The majority of other UF studies did not have imaging data available for all subjects, but instead relied on clinical diagnosis of UFs. As a result, misclassification of UFs within our cohorts should be very low. Additionally, although BioVU participants had a higher mean age than RFTS participants who were primarily in their 20s. It may be that women with UFs in the RFTS cohort represent a group with an early onset of the condition because estimates of age-specific cumulative incidence suggest that many women develop UFs later in their reproductive years.(Laughlin et al., 2010b)

Little is known about UF pathophysiology or genetic risk factors beyond what has been learned from cell culture studies and tumor biology. The GWAS by Cha and colleagues and our findings support that common germline variation may contribute to increased UF risk. When meta-analyzed across all cohorts, including the prior GWAS, the level of statistical significance across all three previously associated GWAS SNPs exceeds the canonical genome-wide threshold for multiple testing. Taken together these data support a consistent effect on risk and suggest that this locus may be functional or in tight LD with the functional SNP. Barriers often faced by UF researchers today include lack of imaging, limited racial diversity in cohorts, and availability of DNA samples. Our study population is unique, as all women included in this replication study had pelvic imaging available to confirm the presence or absence of a UF. Even though only a small number of genetic epidemiology studies have been performed, they have each yielded some important insights into the genetics of UF. Our findings suggest that there is common germline variation that increase risk for UFs among both EA and Japanese; however, further research is necessary in order to assess the role of *BET1L* and *TNRC6B* in other minority groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Final list of SNPs included in UF association analyses among the *Right from the Start* cohort (2001-2012)

rs#	Location	Type
BET1L gene	**Chromosome 11**	
+/- 5 kilobases	187,924-202,382	
rs3741411	189,256	Intron
rs7114102	190,289	Downstream
rs939917	192,547	Downstream
rs11602954	192,856	Downstream
rs2280543*	193,788	3' UTR
rs2280545	194,147	3' UTR
rs1045454	194,228	3' UTR
rs4980319	194,986	3' UTR
rs3782123	195,198	3' UTR
rs7930823	196,767	Intron
rs2293168	201,482	Intron
TNRC6B gene	**Chromosome 22**	
+/- 5 kilobases	38,765,767-39,066,757	
rs7291300	38,770,164	Promoter
rs9611257	38,785,324	Intron
rs6001738	38,789,557	Intron
rs5995802	38,791,365	Intron
rs6001741	38,794,150	Intron
rs11912610	38,796,157	Intron
rs6001743	38,797,954	Intron
rs5995810	38,807,376	Intron
rs7292838	38,809,394	Intron
rs9607685	38,809,757	Intron
rs6001762	38,825,419	Intron
rs11705409	38,826,602	Intron
rs9611265	38,828,439	Intron
rs12157468	38,830,259	Intron
rs9611266	38,830,798	Intron
rs11913462	38,834,510	Intron
rs9611267	38,835,950	Intron
rs17001651	38,841,126	Intron
rs5995814	38,842,688	Intron
rs12628757	38,847,003	Intron
rs6001783	38,854,137	Intron
rs2413611	38,857,804	Intron
rs8140112	38,863,076	Intron
rs2143177	38,865,677	Intron

rs#	Location	Type
rs17323619	38,868,663	Intron
rs11089974	38,873,554	Intron
rs9611286	38,914,908	Intron
rs12628783	38,916,015	Intron
rs8137189	38,929,482	Intron
rs138019	38,941,574	Intron
rs3091342	38,942,102	Intron
rs138022	38,942,982	Intron
rs6001848	38,966,745	Intron
rs5750913	38,970,231	Intron
rs3752513	38,971,926	Intron (boundary)
rs12484776*	38,982,819	Intron
rs12628051	38,984,222	Intron
rs739181	38,986,834	Intron
rs4821940	38,989,519	Intron
rs6001862	39,011,734	Intron (boundary)
rs713898	39,013,786	Intron
rs5995843	39,027,323	Intron (boundary)
rs139909	39,027,527	Intron
rs139910	39,033,834	Intron
rs4821942	39,048,046	Intron
rs139916	39,051,008	3' UTR
rs139921	39,056,708	3' UTR
rs470113	39,059,560	3' UTR
rs12484697	39,066,418	Downstream/Promoter
rs7913069* (nearby SLK)	**Chromosome 10**	
+/- 5 kilobases	105,699,390-105,709,390	
rs7079220	105700137	-
rs2864004	105701838	-
rs11191875	105702778	-
rs7913069*	105704389	-
rs4244255	105709231	Promoter

*Index SNPs

Table 2

Demographic characteristics and their associations with UFs in the *Right from the Start* (2001-2012) and BioVU (2007-present) cohorts

***A. Right from the Start* Cohort**

Characteristic	n	No UFs (n = 552) %	UFs (n = 89) %	OR*	95% CI	
					Lower	Upper
Age						
Less than 25	58	10	2	1.00		Reference
25 to 29	230	38	21	2.52	0.57	11.15
30 to 34	239	37	42	5.12	1.20	21.94
35-49	114	15	35	10.46	2.41	45.46
Greater than or equal to 50	0	0	0	-	-	-
Body mass index						
Underweight (less than 20)	68	11	7	0.74	0.32	1.74
Normal weight (20 to 24.9)	307	48	41	1.00		Reference
Overweight (25 to 29.9)	152	24	21	1.04	0.59	1.83
Obese (30 and above)	114	17	20	1.38	0.77	2.48
Study site						
North Carolina	195	28	54	2.20	1.39	3.47
Tennessee	444	72	46	1.00		Reference
Texas	2	<1	0	-	-	-

B.

Characteristic	n	No UFs (n = 997) %	UFs (n = 997) %	OR	95% CI	
					Lower	Upper
Age						
Less than 25	18	1	<1	1.00		Reference
25 to 29	62	5	1	0.59	0.16	2.22
30 to 34	106	8	3	1.45	0.44	4.74
35-49	508	21	31	5.35	1.73	16.47
50-64	726	28	46	5.71	1.86	17.51
Greater than or equal to 65	527	36	18	1.75	0.57	5.41
Body mass index						

B.

Characteristic	n	No UFs (n = 997) %	UFs (n = 997) %	OR	95% CI	
					Lower	Upper
Underweight (less than 20)	117	7	5	0.75	0.50	1.13
Normal weight (20 to 24.9)	526	29	28	1.00		Reference
Overweight (25 to 29.9)	556	29	31	1.08	0.85	1.36
Obese (30 and above)	670	35	36	1.06	0.84	1.33

n = number, OR = odds ratio, CI = confidence interval

Table 3

Summary of unadjusted index SNP and strongest single locus associations with UFs among the *Right from the Start* and BioVU cohorts

Population	Gene	rs #	MA	MAF		OR	95% CI		P
				No UFs	UF		Lower	Upper	
***RFTS EA*[1]**	*Nearby SLK*	rs11191875	T	0.02	0.05	2.78	1.24	6.26	0.014
		rs7913069*	T	0.01	0.01	0.42	0.05	3.20	0.400
	BET1L	rs939917	T	0.34	0.50	1.86	1.12	3.07	0.016
		rs2280543*	T	0.04	0.02	0.55	0.20	1.56	0.262
	TNRC6B	rs11089974	T	0.20	0.26	1.46	1.01	2.10	0.046
		rs12484776*	G	0.20	0.27	1.48	1.03	2.13	0.035
		rs4821942	A	0.20	0.28	1.52	1.06	2.18	0.024
BioVU EA									
	Nearby SLK	rs7913069*	T	0.01	0.02	1.18	0.71	1.97	0.527
	BET1L	rs2280543*	T	0.05	0.04	0.68	0.51	0.92	0.013
	TNRC6B	rs12484776*	G	0.20	0.23	1.17	1.00	1.36	0.050

MA = major allele, MAF = minor allele frequency, OR = odds ratio, CI = confidence interval

Highlighted results indicate $p \leq 0.05$

[1] European Americans are all non-Hispanic

* Index SNP from previous GWAS

Table 4

Unadjusted SNP and UF associations from meta-analyses across *Right from the Start*, BioVU, and a previously published GWAS of a Japanese population[†]

Meta-Analysis Populations	Gene	MA	rs #	OR	SE	Q	I	P
RFTS EA and BioVU	*Nearby SLK*	T	rs7913069[*]	1.11	0.25	0.33	0	0.682
	BET1L	T	rs2280543[*]	0.67	0.15	0.70	0	6.9×10^{-3}
	TNRC6B	G	rs12484776[*]	1.21	0.07	0.24	28.37	8.7×10^{-3}
RFTS EA, BIOVU EA, and Prior Japanese GWAS [†]	*Nearby SLK*	T	rs7913069[*]	1.58	0.08	0.21	36.01	7.45×10^{-8}
	BET1L	T	rs2280543[*]	0.66	0.07	0.92	0	3.89×10^{-9}
	TNRC6B	G	rs12484776[*]	1.26	0.04	0.38	0	1.33×10^{-8}

OR = odds ratio, SE =standard error, Q = p value for the Cochrane's Q statistic, $I = I^2$ heterogeneity index (0-100)

Highlighted results indicate $p \leq 0.05$

[*] Index SNP from previous GWAS. (30)